

Appl. No. 09/863,693
Amendment dated June 9, 2005
Reply to Office Action of March 16, 2004

This listing of claims will replace all prior versions and listings of claims in the application.

Listing of Claims:

1-87. canceled

88. (new) A method of preparing a bispecific antibody comprising:

(i) culturing a host cell comprising one or more nucleic acids encoding:

(a) a first polypeptide comprising a first multimerization domain and a first antibody variable heavy chain domain specific for a first antigen;

(b) a second polypeptide comprising a second multimerization domain and a second antibody variable heavy chain domain specific for a second antigen; and

(c) a variable light chain polypeptide that has 100% amino acid sequence identity to a variable light chain specific for the first antigen and a variable light chain specific for the second antigen;

wherein the variable light chain polypeptide and the first antibody variable heavy chain domain form an antigen binding domain for the first antigen, and the variable light chain polypeptide and the second antibody variable heavy chain domain form an antigen binding domain for the second antigen, wherein the first and second antigens differ from one another, and the first and second multimerization domains interact to form the bispecific antibody; and

(ii) recovering the bispecific antibody from the host cell culture.

89. (new) The method of claim 87, wherein the first polypeptide and the second polypeptide each further comprise an antibody constant domain.

90. (new) The method of claim 89, wherein the antibody constant domain is a C_H3 domain.

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91. (new) The method of claim 88, wherein the first or the second multimerization domain comprises an immunoglobulin sequence, a leucine zipper, a hydrophobic region, a hydrophilic region, a protuberance, a cavity, or a free thiol which forms an interpolypeptide disulfide bond.

92. (new) A host cell comprising one or more nucleic acids encoding:

(a) a first polypeptide comprising a first multimerization domain and a first antibody variable heavy chain domain specific for a first antigen;

(b) a second polypeptide comprising a second multimerization domain and a second antibody variable heavy chain domain antibody specific for a second antigen; and

(c) a variable light chain polypeptide that has 100% amino acid sequence identity to a variable light chain domain specific for the first antigen and a variable light chain domain specific for the second antigen;

wherein the variable light chain polypeptide and the first antibody variable heavy chain domain form an antigen binding domain for the first antigen, and the variable light chain polypeptide and the second antibody variable heavy chain domain form an antigen binding domain for the second antigen, wherein the first and second antigens differ from one another, and the first and second multimerization domains interact to form the bispecific antibody.

93. (new) The host cell of claim 92, wherein the host cell is a mammalian cell.

94. (new) A method of preparing a bispecific antibody comprising:

(i) culturing a host cell comprising one or more nucleic acids encoding:

(a) a first polypeptide comprising a first multimerization domain and a first antibody variable heavy chain domain specific for a first antigen;

(b) a second polypeptide comprising a second multimerization domain and a second antibody variable heavy chain domain specific for a second antigen;

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(c) a first variable light chain polypeptide specific for the first antigen and a second variable light chain polypeptide specific for the second antigen; wherein each of the first and second variable light chain polypeptides has at least 3 CDRs and has at least 98% amino acid sequence identity to the other variable light chain polypeptide and differs only at amino acid positions outside of the CDRs; and

wherein a first antigen binding domain is formed by pairing either the first or second antibody variable light chain polypeptide with the first antibody variable heavy chain variable domain and the second antigen binding domain is formed by pairing the first or second variable light chain polypeptide with the second variable heavy chain domain; wherein the first and second multimerization domains interact to form a bispecific antibody; and

(ii) recovering the bispecific antibody from the host cell culture.

95. (new) The method of claim 94, wherein the first polypeptide and the second polypeptide each further comprise an antibody constant domain.

96. (new) The method of claim 95, wherein the antibody constant domain is a C_H3 domain.

97. (new) The method of claim 94, wherein the first or the second multimerization domain comprises an immunoglobulin sequence, a leucine zipper, a hydrophobic region, a hydrophilic region, a protuberance, a cavity, or a free thiol which forms an interpolypeptide disulfide bond.

98. (new) The method of claim 94, wherein the first variable light chain polypeptide has at least 99% amino acid sequence identity to the second variable light chain polypeptide.

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99. (new) The method of claim 94, wherein the first and the second variable light chain polypeptides have the same sequence.

100. (new) A host cell comprising one or more nucleic acids encoding:

- (a) a first polypeptide comprising a first multimerization domain and a first antibody variable heavy chain domain specific for a first antigen;
- (b) a second polypeptide comprising a second multimerization domain and a second antibody variable heavy chain domain specific for a second antigen;
- (c) a first variable light chain polypeptide specific for the first antigen and a second variable light chain polypeptide specific for the second antigen; wherein each of the first and second variable light chain polypeptides has at least 3 CDRs and has at least 98% amino acid sequence identity to the other variable light chain polypeptide and differs only at amino acid positions outside of the CDRs; and

wherein a first antigen binding domain is formed by pairing either the first or second antibody variable light chain polypeptide with the first antibody variable heavy chain domain and the second antigen binding domain is formed by pairing the first or second variable light chain polypeptide with the second variable heavy chain domain; wherein the first and second multimerization domains interact to form a bispecific antibody.

101. (new) The host cell of claim 100, wherein the host cell is a mammalian cell.

102. (new) A method of preparing a bispecific antibody comprising:

- (i) culturing a host cell comprising one or more nucleic acids encoding:
 - (a) a first polypeptide comprising a first multimerization domain and a first antibody variable heavy chain domain specific for a first antigen;
 - (b) a second polypeptide comprising a second multimerization domain and a second antibody variable heavy chain domain specific for a second antigen;

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(c) a variable light chain polypeptide, wherein the variable light chain polypeptide has at least 3 CDRs and has at least 98% amino acid sequence identity to a first variable light chain domain specific for the first antigen or to a second variable light chain domain specific for the second antigen or to both of the first and second variable light chain domains, the variable light chain polypeptide differs from the first or second variable light chain domains or both only at amino acid positions outside of the CDRs;

wherein the variable light chain polypeptide and the first variable heavy chain domain form an antigen binding domain for the first antigen, and the variable light chain polypeptide and the second variable heavy chain domain form an antigen binding domain for the second antigen, and the first and second multimerization domains interact to form a bispecific antibody; and

(ii) recovering the bispecific antibody from the host cell culture.

103. (new) The method of claim 102, wherein the first polypeptide and the second polypeptide each further comprise an antibody constant domain.

104. (new) The method of claim 103, wherein the antibody constant domain is a C_H3 domain.

105. (new) The method of claim 102, wherein the first or the second multimerization domain comprises an immunoglobulin sequence, a leucine zipper, a hydrophobic region, a hydrophilic region, a protuberance, a cavity, or a free thiol which forms an interpolypeptide disulfide bond.

106. (new) The method of claim 102, wherein the first variable light chain polypeptide has at least 99% amino acid sequence identity to the first and second variable light chain polypeptide.

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107. (new) The method of claim 102, wherein the first and the second variable light chain domains have the same sequence.

108. (new) A host cell comprising one or more nucleic acids encoding:

- (a) a first polypeptide comprising a first multimerization domain and a first antibody variable heavy chain domain specific for a first antigen;
- (b) a second polypeptide comprising a second multimerization domain and a second antibody variable heavy chain domain specific for a second antigen;
- (c) a variable light chain polypeptide, wherein the variable light chain polypeptide has at least 3 CDRs and has at least 98% amino acid sequence identity to a first variable light chain domain specific for the first antigen or to a second variable light chain domain specific for the second antigen or to both of the first and second variable light chain domains, and the variable light chain polypeptide differs from the first or second variable light chain domains or both only at amino acid positions outside of the CDRs; wherein the variable light chain polypeptide and the first variable heavy chain domain form an antigen binding domain for the first antigen, and the variable light chain polypeptide and the second variable heavy chain domain form an antigen binding domain for the second antigen, and the first and second multimerization domains interact to form a bispecific antibody.

109. (new) The host cell of claim 108, wherein the host cell is a mammalian cell.